*Serangga 21(1): 51-59*
*ISSN 1394-5130 © 2016, Centre for Insects Systematic,*
*Universiti Kebangsaan Malaysia*

# THE LOCALIZATION OF LUCIFERASE IN *PTEROPTYX TENER* (COLEOPTERA: LAMPYRIDAE) LIGHT ORGAN

**Nur Khairunnisa S., Nurul Wahida O. and Norela S.**
Centre for Insects Systematic, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
*Corresponding author: vozela@ukm.edu.my*

## ABSTRACT

Firefly is famously known for the light emission from its abdominal light organ. Light produce by firefly is known as bioluminescence. Luciferase is the enzyme that catalyze the light emitting reaction that produce bioluminescence. Haematoxylin and Eosin staining were used to describe the histological structure of the light organ. Localization of luciferase was discovered by immunohistochemical staining with polyclonal anti-luciferase and DAB chromogen as secondary antibody. Microphotography of light organ tissues was taken by Carl Zeiss Axio Scope A1 photomicroscope. This study revealed that the luciferase enzyme located at the photocyte cytoplasm of photogenic layer. This finding is an initial step to fully understand the regulation of synchronous light production in *P. tener*.

**Keywords**: *Pteroptyx tener*, Lampyridae, firefly, Luciferase, light organ

## ABSTRAK

Kelip-kelip terkenal dengan kebolehan mengeluarkan cahaya daripada organ cahaya yang terletak pada abdomennya. Cahaya yang dihasilkan oleh kelip-kelip ini dikenali sebagai bioluminasi dan luciferase adalah enzim yang berfungsi untuk memangkin proses penghasilan bioluminasi. Tisu organ cahaya diwarnakan mengikut kaedah pewarnaan Hematoksilin dan Eosin (H&E) untuk pencerapan struktur histologi organ cahaya. Manakala pewarnaan imminohistokimia menggunakan poliklonal anti-luciferase pula digunakan untuk mengenal pasti kedudukan enzim luciferase yang terdapat pada organ cahaya. Sampel tisu yang telah siap diproses diperhatikan dan foto direkodkan menggunakan mikroskop Carl Zeiss Axio Scope A1. Kajian ini telah menunjukkan lokasi enzim luciferase terletak pada sitoplasma sel photosit yang terdapat pada lapisan photogenik.

**Kata kunci**: *Pteroptyx tener*, Lampiridae, kelip-kelip, luciferase, organ cahaya

## INTRODUCTION

*Pteroptyx tener* is an Asian firefly and one of a few fireflies' species in this world that flash synchronously. This species congregate mangrove trees and the male will flash light synchronously to attract female for mating (Case 1980; Copeland & Moiseff 1997; Wan Faridah Akmal Wan Jusoh 2010). The synchronous light control mechanism this species is yet to understand. Before cellular mechanism for controlling the synchronously light reaction can be completely worked out it is necessary to know the subcellular location of fluorescent compound involved.

Firefly bioluminescence is a spectacular visual display of insect. The light production is a result of chemical reaction that occurs in the special organ known as light organ. Luciferase is an enzyme responsible for the bioluminescence reaction in fireflies that catalyzed the oxidation of organic substrate, luciferin, with molecular oxygen in the presence of ATP and Mg2+ (Mcelroy1969, Oba et al. 2012; Viviani 2002; White et al. 1963)

## MATERIALS AND METHODS

Fireflies for this study were collected from the stretch of the Selangor River in Kampung Kuantan, Kuala Selangor, Malaysia. The sampling were done after sunset between 1900-2000 hours from January to May 2014. Adult fireflies were captured alive during sampling using the sweep net.

### Histology structure

The light organ (both at 6th and 7th abdominal segments) were extracted and immersed in 10% formalin fixative overnight. After fixation, the tissues were put through a series of ethanol (70%, 80%, 90% and 100%) to dehydrate then followed by xylene immersion. Tissues were infiltrated with paraffin wax and embedded. Blocks of waxed tissues were then sectioned by LEICA RM2245 microtome and set to cut at 4 µm. Four to five sections were placed on a slide and dried overnight. Sectioned tissues were then stained according to Hematoxylin & Eosin (H&E) reagent method (Fischer 2008). Slides were mounted with DPX mounting medium. Microphotography was taken by Carl Zeiss Axio Scope A1 photomicroscope with iSolutionLite v1.0 software for observation.

**Luciferase localization**

To discover the location of luciferase, sections of *P. tener* light organ were treated with a polyclonal anti-luciferase and the bound antibody was observed by DAB chromogen labelling. Tissue preparation procedure were same as above method but for immunohistochemical studies the tissue sections were attached on poly L Lysine coated slides. The section were dewaxing in xylene before dehydrated through an ethanol series followed by water. Heat Induced Epitope Retrieval (HIER) procedure were used for antigen retrieval treatment. Tissue section were incubated in Tris-EDTA antigen retrieval buffer at 95°C for 30 minutes before the sections were rinsed with PBT+N (PBT + 5% normal goat serum). Nonspecific antibody binding were blocked by incubate the sections in H2O2 for 5 minutes then incubated in diluted (1: 1000) primary antibody for 30 minutes. The polyclonal Anti-Firefly Luciferase antibody (ab21176) used was obtained from ABCAM. After rinse with PBT+N, sections were incubated in HRP conjugated secondary antibody (dilution 1:300) for 30 minutes. Before the section incubated in DAB chromogen for 10 minutes, the PBT+N were used to wash off antibodies for several times. Then the tissues were counterstained with hematoxylin, dehydrated in series alcohol, cleared with xylene and mounted with DPX mounting medium. Slides were observed with Carl Zeiss Axio Scope A1 photomicroscope and iSolutionLite v1.0 software.

## RESULTS AND DISCUSSIONS

*Pteroptyx tener* light organ is composed of two distinct layer, photogenic layer and reflective layer (Fig 1a-b). Photogenic layer is where the light production occur. Figure 1, showed there are several elements in photogenic layer. There are photocytes, differentiated zones, cylinder, trachea, and tracheal end cells. Photocyte contains photocyte granules, centrally located nucleus and differentiated zone. Differentiated zone is a region of photocyte cytoplasm that lacked granules and located adjacent

to cylinder (Fig 1d). Cylinder consist of trachea and extend vertically through the photogenic layer (Fig 1c). According to Buck (1948), the trachea branch extensively to form tracheole between photocyte. The reflector layer is composed of irregular shaped of cells packed with circular granules. Studies done by Goh [8] showed that this tiny granules are uric acid granules and this layer capable of reflecting bioluminescence.

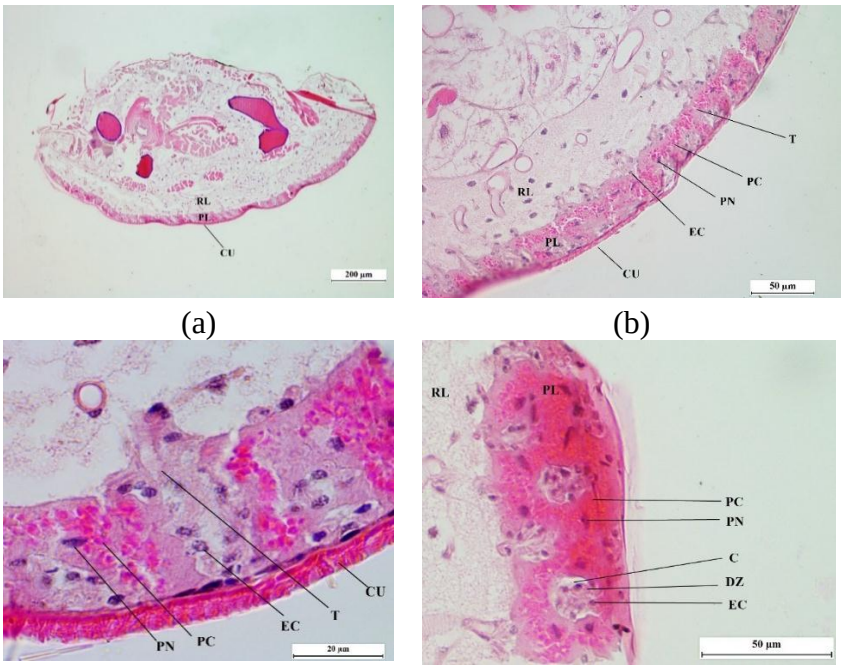


Figure 1 Histological structure of adult male light organ (H & E staining). a) Cross section of whole abdomen (100x) b) (200x) & c) vertical section of light organ (400x) d) horizontal section of light organ (1000x). C, cylinder; CU, cuticle; DZ, differentiate zone; EC, tracheal end cell; PC, photocyte; PL, Photogenic layer; PN, photocyte nucleus; RL, reflector layer; T,trachea.

In photogenic layer, immunohistochemical staining showed photocytes were clearly labelled with DAB chromogen, particularly in photocyte cytoplasm. This shows that there is high concentration of luciferase in the cell cytoplasm. However, others elements in photogenic layer, differentiated zone, tracheal end cell and cylinders were lacked DAB chromogen. This showed the light production of *P. tener* only occurred in photocyte cytoplasm and the luciferase enzyme were maybe synthesized by photocyte cell.

In contrast with the previous studies by Smalley (1980), reported that, are also noticeable fluorescence material in the reflector layer cells located at the border of photogenic layer. In this studies, the luciferase were absent in reflector layer. The absent of luciferase enzyme in this layer showed that this layer is not involved in the light production.

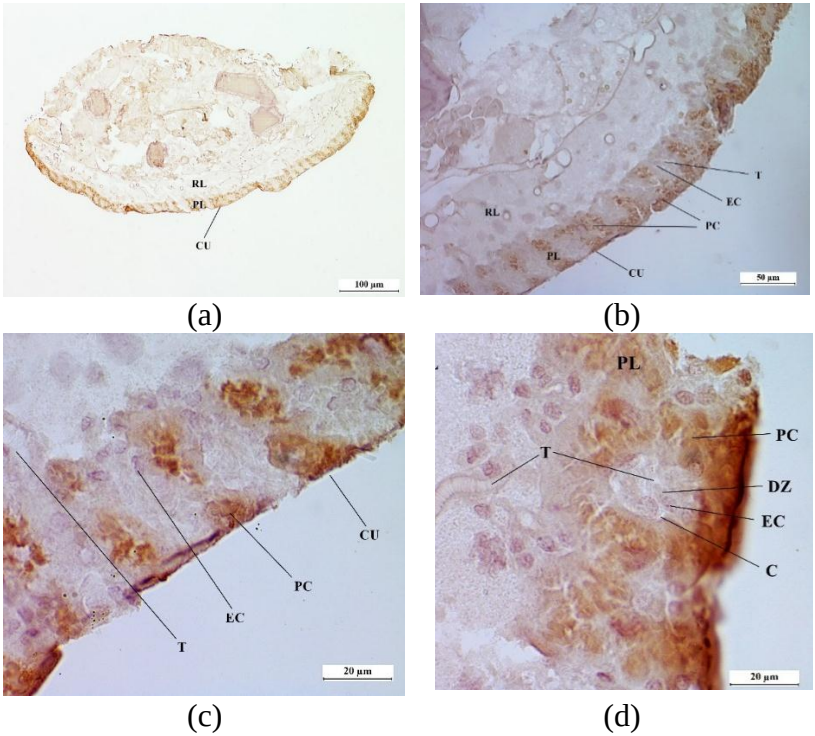


Figure 2 Sections of adult male light organ treated by immunohistochemistry to localize the luciferin. a) Cross section of whole abdomen (100x) b) (200x) & c) vertical section of light organ (400x) d) horizontal section of light organ (1000x). C, cylinder; CU, cuticle; DZ, differentiate zone; EC, tracheal end cell; PC, photocyte; PL, Photogenic layer; PN, photocyte nucleus; RL, reflector layer; T, trachea.

## CONCLUSION

The light production of *P. tener* was taken place in photocyte cytoplasm without the involvement of reflective layer as found in previous studies on *Photuris* fireflies.

## ACKNOWLEDGEMENT

This research was funded by grants LAUREATE-2013-002.The author would like to thanks to Faculty Science of Technology, UKM for the facilities. Mrs Nur Maya Sabrina and Mr Ammar, PPUKM research officer for helpful advice and technical assistance.

## REFERENCES

Buck, J. B. 1948. The Anatomy and Physiology of the Light Organ in Fireflies. *Annals of the New York Academy of Sciences* 49(3): 397-485.

Case, J. F. 1980. Courting Behavior in a Synchronously Flashing, Aggregative Firefly, *Pteroptyx tener*. *The Biological Bulletin* 159(3): 613-625.

Copeland, J. & Moiseff, A. 1997. The Effect of Flash Duration and Flash Shape on Entrainment in *Pteroptyx malaccae,* a Synchronic Southeast Asian Firefly. *Journal of Insect Physiology* 43(10): 965-971.

Fischer, A. H., Jacobson, K. A., Rose, J. & Zeller, R. 2008. Hematoxylin and Eosin Staining of Tissue and Cell Sections. *Cold Spring Harbor Protocols* 2008(5): pdb. prot4986.

Goh, K-S., Sheu, H-S., Hua, T-E., Kang, M-H. & Li, C-W. 2013. Uric Acid Spherulites in the Reflector Layer of Firefly Light Organ. *PLoSONE* 8(2): e56406.

Mcelroy, W., Seliger, H. & White, E 1969. Mechanism of Bioluminescence, Chemi-Luminescence and Enzyme Function in the Oxidation of Firefly Luciferin*, †. *Photochemistry and Photobiology* 10(3): 153-170.

Oba, Y., Yoshida, M., Shintani, T., Furuhashi, M. & Inouye, S. 2012. Firefly Luciferase Genes from the Subfamilies Psilocladinae and Ototretinae (Lampyridae, Coleoptera). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 161(2): 110-116.

Smalley, K., Tarwater, D. & Davidson, T. 1980. Localization of Fluorescent Compounds in the Firefly Light Organ. *Journal of Histochemistry & Cytochemistry* 28(4): 323-329.

Viviani, V. 2002. The Origin, Diversity, and Structure Function Relationships of Insect Luciferases. *Cellular and Molecular Life Sciences CMLS* 59(11): 1833-1850.

Wan Faridah Akmal Wan Jusoh, N. R. H. a. Z. Z. I. 2010. Distribution and Abundance of *Pteroptyx* Fireflies in Rembau-Linggi Estuary, Peninsular Malaysia. *Environment Asia* 3 (Special Issue): 56-60.

White, E. H., Mccapra, F. & Field, G. F. 1963. The Structure and Synthesis of Firefly Luciferin. *Journal of the American Chemical Society* 85(3): 337-343.